Studying rules governing microbial communities

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ESPP2 Environmental Stress Pathway Project

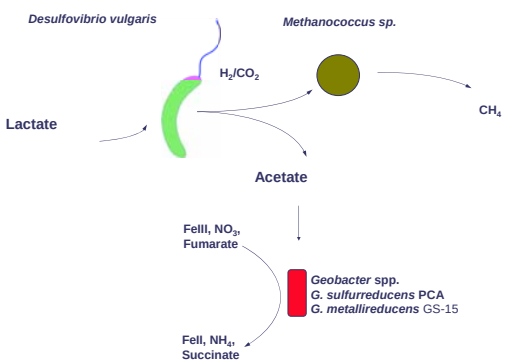
DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

VIMSS Virtual Institute for Microbial Stress and Survival — http://vimss.lbl.gov/

INTRODUCTION

One objective of the Virtual Institute for Microbial Stress and Survival (VIMSS) and the Environmental Stress Pathway Project (ESPP) is to determine the genetic and physiological bases for cooperative and competitive interactions among environmental microbial populations of relevance to the DOE. Understanding the behavior of biological communities presents tremendous challenges because of the complex network of diverse interactions among species. A class of communities of particular interest from ecological, geological, and engineering perspectives is represented by microbial communities that thrive in oxygen-free (anoxic) environments. These communities are vital components in numerous environments including freshwater sediments, the subsurface, guts of insects and animals, and wastewater treatment plants. They therefore play a significant role in global cycling of carbon and other biogenic elements. Our prior research established the feasibility of working with a simple two-tier food web composed of two species, each species occupying a distinct trophic position: *Desulfovibrio vulgaris* syntrophically coupled to the hydrogen consuming *Methanococcus maripaludis*. We are now developing more complex assemblies to examine competitive and cooperative interactions, and factors contributing to community stability. We are using organisms having fully sequenced genomes (*Desulfovibrio vulgaris, Geobacter sulfurreducens* PCA, *Geobacter metallireducens* GS-15 and *Methanococcus maripaludis*) to construct different tri-cultures and develop tools to monitor community composition. *G. sulfurreducens* and *G. metallireducens* consume acetate and use alternative electron acceptors, including nitrate, fumarate and iron.

Figure 1. Interactions in model microbial communities



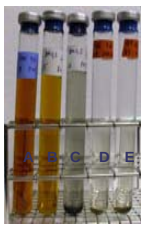
Studying model artificial communities we address number of ecologically important questions

- Does biomass yield of a community correlate with overall free energies of reaction?
- Does biomass yield of a of multi-trophic level community correlate with free energy of the terminal metabolic reaction? The initial electron donor may vary, but the terminal electron accepting reaction remains constant (i.ei, methanogenesis from hydrogen and carbon dioxide).
- What is the distribution of available free energy among species in a multi-trophic level microbial community?
- What parameters (physicochemical and biological) determine the minimum free energy sufficient for growth of multi- trophic level microbial communities?

RESULTS

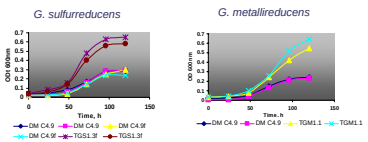
The ecological relevance of alternative electron acceptor availability in constructed microbial food webs is being examined by measuring growth kinetics and yields in relationship to thermodynamic predictions. Initial studies have shown that lactate-grown tri-cultures of *D. vulgaris* and *M. maripaludis* combined with either *G. sulfurreducens* (plus fumarate (Figure 3 and 4) or iron citrate (Figure 2) or *G. metallireducens* (plus nitrate (Figure 3) or iron citrate (Figure 2) evolved both methane and hydrogen at different stages of growth in batch culture (Figure 4).

Figure 2. Methanogenic tricultures of *D. vulgaris, M. maripaludis* and *G. sulfurreducens* and *G.metallireducens* reduce iron citrate using lactate as an electron donor

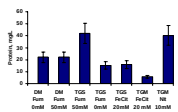


A – coculture of *D. vulgaris, M. maripaludis* reduced iron citrate very slowly;
B & C – triculture of *D. vulgaris, M. maripaludis* and *G. sulfurreducens or G metallireducens* rapidly reduced the iron citrate;
D and E – pure culture of *G. sulfurreducens* reduced iron in B3 medium amended with acetate

Figure 3. Methanogenic tri cultures of *D. vulgaris, M. maripaludis* and *G. sulfurreducens* and *G. metallireducens* reduce fumarate and nitrate



Biomass yield (protein mg/L) at the time when lactate was consumed and growth ceased



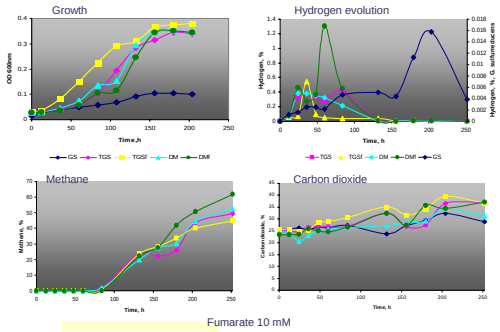
DM – *D. vulagaris* and *M. maripaludis* coculture;
TGS - *D. vulagaris*, *G. sulfurreducens* PCA and *M. maripaludis* triculture;
TGM - *D. vulagaris* , *G. metallireducens* GS-15 and *M. maripaludis* triculture; f - presence of fumarate

MATERIALS and METHODS

Cultures were grown on a B3o medium in 25 ml Balch tubes at 30 psi with either a 80%N2: 20%CO2 or 80%H2:20% CO2 gas mixture in the headspace volume of approximately 15 ml. The basal B3o medium (pH 7.2) contained (per liter): 2.5g NaCl, 5.5 g MgCl2•6H20, 0.1g CaCl2•2H20, 0.5g NH4Cl, 0.1g KCl, 1.4g Na2SO4, 25mM NaHCO3, 5.75mM K2HPO4, 0.001g resazurine, 0.078g Na2S · 9 H2O, 1ml Thauer's vitamins of (containing per liter 0.02g biotin, 0.02g folic acid, 0.1g pyridoxine HCl, 0.05g thiamine HCl, 0.05g riboflavin, 0.05g nicotinic Acid, 0.05g DL pantothenic acid, 0.05g *p*-aminobenzoic Acid, 0.01g vitamin B12), 1ml of trace minerals (per liter: 1.0g FeCl2•4H2O, 0.5g MnCl2•4H2O, 0.3g CoCl2•4H2O, 0.2g ZnCl2, 0.05g Na2MoO4•4H2O, 0.02g H3BO3 , 0.1g NiSO4•6H2O, 0.002g CuCl2•2H2O, 0.006g Na2SeO3•5H2O, 0.008g Na2WO4•2H2O). This basal medium was amended with lactate and 10mM or 50 mM of fumarate; 20 mM of Fe citrate or 10 mM nitrate as electron acceptor .

The concentration of organic acids and inorganic ions (sulfate, phosphate) in culture fluids were determined using a Dionex 500 system equipped with an AS11HC column. In some cases the concentration of organic acids was also measured on an HPLC equipped with a HPX 78 (Bio-Rad) column. Hydrogen concentrations were determined with a RGD2 Reduction Gas Detector (Trace Analytical) with 60/80 MOLE SIEVE 5A column (6' X 1/8'') with N2 as carrier gas. The concentration of methane and carbon dioxide was measured on a GC equipped with a TCD and "80/100 HAYESEP Q" column (6' X 1/8'') with helium as carrier gas.

Figure 4. Methanogenic co-cultures and tri cultures evolve different amount of hydrogen in the presence and absence of fumarate



Free energy changes of reactions involved in growth of tri cultures of *D. vulgaris, M. maripaludis and Geobacter* species

Electron donor	Electron acceptor	ΔGo' KJ	Reference
lactate	No e	-3.96	Noguera et al., 1998
lactate	coculture	-185	This study
acetate	fumarate	-244	This study
acetate	nitrate	-500	Lovley & Phillips, 1988
acetate	FeIII	-814	Lovley & Phillips, 1988
lactate	fumarate,triculture	-671	This study
lactate	nitrate, triculture	-1183	This study
lactate	FeIII, triculture	-1811	This study

CONCLUSIONS

Initial studies have shown that lactate-grown tri-cultures of *D. vulgaris* and *M. maripaludis* combined with either *G. sulfurreducens* (plus fumarate or iron citrate) or *G. metallireducens* (plus nitrate or iron citrate) evolved both methane and hydrogen at different stages of growth in batch culture.

Tri-cultures with *G. sulfurreducens* growing with fumarate and tri culture with *G. metallireducens* growing with nitrate demonstrated comparable growth rates and biomass yields despite differences in predicted total free energy: **-671** kJ for fumarate and **-1183** kJ for nitrate.

Methanogenic co-cultures and tri cultures evolved different amount of hydrogen in the presence and absence of fumarate reflecting changes in the metabolic activities of community members.

ACKNOWLEDGEMENT

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